**VICH,** International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.

**WORKING GROUP :** **Biologicals Quality Monitoring.**

**TOPIC**: ***Test on the absence of extraneous agents***

# *R E V I S E D  F O R M A T*

# DRAFT TEXT

GUIDELINE FOR THE TESTS TO DETERMINE THE PRESENCE OF EXTRANEOUS AGENTS IN VETERINARY VACCINES.

## A. MAMMALIAN VIRAL VACCINES PRODUCED IN ESTABLISHED CELL-LINES.

*Part I. Tests to determine the presence of extraneous viruses.*

## Date : JANUARY 2000, VS 2000/01

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## 1.INTRODUCTION.

### 1.1 Objective of the guideline.

It is important that biological products for veterinary use are free of contaminants, notably viral agents. Potential sources of contamination are the viral and bacterial strains used for the production of the active ingredient(s) and the starting materials of animal origin used in the production of the active ingredient and / or in the assembly of the finished product. Consequently it is necessary to demonstrate that extraneous agents are not present in biological products nor in the starting materials required for their manufacture, through the use of accepted testing procedures and sampling methods and subject to the limitations of the test.

The purpose of the guideline is to provide a description of the test methods to detect the presence of extraneous agents which shall be undertaken on all materials of animal origin used in the production of veterinary immunological products for use in mammals. It shall also provide precise information on the method and conditions of the tests to determine the absence of extraneous viruses in these substances.

### 1.2. Background.

The materials used in the manufacture of biological products for veterinary use can be divided into two main categories:

1. Viral strains and cell substrates used in the production of the active ingredient(s).

2. Starting materials of animal origin used in the production of the active ingredients and/or in the assembly of the finished product.

Restrictions may be placed by regulatory authorities upon the use of starting materials of animal origin to minimise the risk associated with pathogens that may be potentially present in these materials e.g.: their use is not generally acceptable except when they are sterilised by a suitable, validated method.

Where the use of such substances has been shown to be essential and sterilisation not possible, it will normally be required to test and monitor the source animals for freedom from infectious agents and/or to test these substances for the absence of contaminants. [ *In the case of inactivated vaccines, the method used for inactivation of the vaccine strain may also be validated for inactivation of possible contaminants from substances of animal origin.* ]

Present methods of testing for extraneous agents of substances of animal origin are described in the European Pharmacopoeia monograph 62 (1995) and in the Code of Federal Regulations 9CFR 113 and the OIE Manual of Standards for Diagnostic Tests and Vaccines.

[ *The use of experimental animals in tests shall be minimised and if required its necessity shall be justified* ]

### 1.3. Scope of the guideline.

The scope of the guideline is to provide guidance on the methods to determine the presence of extraneous viruses in veterinary viral vaccines for mammals manufactured in established cell lines. The test methods are intended for the test on the finished product and on starting materials of animal origin used in the manufacture of the vaccine e.g. seeds and cell stocks.

## 2. GUIDELINE FOR TESTING FOR PRESENCE OF EXTRANEOUS VIRUSES IN MAMMALIAN VIRAL VACCINES.

### 2.1. Test for the presence of extraneous viruses in virus seeds

#### 2.1.1. *General* .

Normally , whenever possible, the virus seeds are prepared and maintained in accordance with the seed lot principle.

It is normally required that a viral seed materials are tested to the extent possible for the presence of extraneous agents. Tests should be carried out for possible contaminating viruses that may originate from:

- the source species of the material.
- from those animal species to which the seed material may have been exposed to e.g. during passaging.

and also

- for the presence of possible contaminating viruses that may occur in the animal species for which the finished product is intended.

If during one or more of the preparatory stages of the testing for extraneous agents an additional process is used e.g. adsorbtion it will be necessary to demonstrate that this process does not affect the sensitivity of the test and the testresults.

#### *2.1.2. Samples*

The samples shall originate from well-defined seedlots only.

The Master Seed Virus shall - when necessary - be neutralised with a monoclonal or polyclonal antiserum containing high levels of neutralising antibody to the virus present in the Master Seed Virus.
This antiserum shall be prepared with an antigen that :

- is not derived from any passage level of the virus isolate giving rise to the Master Seed Virus.
- has a different passage history.

The antiserum shall be free of antibodies against those viruses for which presence the Master Seed is tested.

*2.1.3. Substrates*

The substrates used for testing the absence of extraneous agents shall consist of at least three different types of sensitive cells and include at least:

- primary cells of the source species;
- cells sensitive to viruses pathogenic for the species for which the vaccine is intended;
- cells sensitive to pestiviruses. The sensitivity of these cells shall be demonstrated by the use of positive controls e.g. Bovine Viral Diarrhoea virus.

Where relevant, other cells or substrates e.g. eggs, sensitive to other relevant viruses that may potentially be present in the virus seed ,as a result of passaging the virus strain in cells of other animal species shall be used.

*2.1.4. Test method*

2.1.4.1. Preparation of the substrate cells.

The monolayers shall be prepared in suitable culture vessels.

The monolayers to be used in the test shall cover an area of at least 75 $cm^2$ and shall be prepared and maintained using medium and additives, and grown under similar conditions – as far as is feasible - to those used for the preparation of the vaccine. At least the culture-media, the cell-system and incubation temperature shall be mimicked.

2.1.4.2. Inoculum.

A quantity of virus, equivalent to that present in 10 doses of vaccine is inoculated on at least 8 monolayers of suitable cells with a surface of 20 $cm^2$ each.

> **Note:** The number of monolayers depends on the number of different types of erythrocytes used for haemadsorbtion testing.

2.1.4.3. Procedure.

2.1.4.3.1. General.

All cell cultures are maintained for at least 21(28) days, during which at least 2 subcultures are made at 7 days intervals, unless the cells do not survive for this length of time, when the subcultures shall be made on the latest day possible.

They are examined as follows:

- All cultures are observed regularly *( daily ??)* for cytopathogenic effect.
- At the end of the last subculture
    - 2 monolayers are stained and examined microscopically for cytopathogenic effect, inclusion bodies etc.
    - the other monolayers are examined for haemadsorbtion.

In addition the absence of specific extraneous agents shall be determined by means of immunofluorescent antibody technique using monolayers taken at least 7 days after the initiation of the last subculture, using appropriate controls.

2.1.4.3.2. Examination of the cultures for cytopathogenic viruses.

Two monolayers of at least 6 $cm^2$ each are stained with an appropriate cytological stain. The entire area of each stained monolayer is examined for any inclusion bodies, abnormal numbers of giant cells or any other lesion indicative of a cellular abnormality which might be attributable to a contaminant. The method described in the EP would be acceptable.

2.1.4.3.3. Examination for haemadsorbent viruses.

Monolayers totalling at least 70 $cm^2$ are washed several times with an appropriate buffer. A sufficient volume of a suspension of appropriate red blood cells, but at least including chicken and guineapig erythrocytes, are added to cover the surface of the monolayer evenly. After incubation for 30 minutes at +4º C and incubation for 30 minutes at 20-25 ºC and, where necessary under other appropriate conditions, cells are examined for the presence of haemadsorption. The method described in the EP would be acceptable.

2.1.4.3.4. Examination for specified viruses.

For viruses, which are unlikely to be detected by the general tests described above, specific test methods must be applied. These are indicated under the heading “Specific tests” in the relevant tables.

**2.3. SPECIFIC TEST METHODS**

**2.3.1. Preparation of the substrate cells.**

The monolayers shall be prepared in *suitable culture vessels*.

The monolayers to be used in the test shall cover an area of at least 75 $cm^2$ and shall be prepared and maintained using medium and additives, and grown under similar conditions – as far as is feasible - to those used for the preparation of the vaccine. At least the culture-media, the cell-system and incubation temperature shall be mimicked.

The monolayers are maintained in culture for a total of at least 28 days. At least two subcultures are made at 7-day intervals, unless the cells do not survive for this length of time, when the subcultures are made on the latest day possible.

**2.3.2. Examination of the cultures for cytopathogenic viruses.**

Two monolayers of at least 6 $cm^2$ each are stained with an appropriate cytological stain. The entire area of each stained monolayer is examined for any inclusion bodies, abnormal numbers of giant cells or any other lesion indicative of a cellular abnormality which might be attributable to a contaminant. The method described in the EP would be acceptable.

2.3.3. Examination for haemadsorbent viruses.

Monolayers totalling at least 70 $cm^2$ are washed several times with an appropriate buffer. A sufficient volume of a suspension of appropriate red blood cells,but at least including chicken and guineapig erythrocytes, are added to cover the surface of the monolayer evenly. After incubation for 30 minutes at +4º C and incubation for 30 minutes at 20-25 ºC and ,where necessary under other appropriate conditions, cells are examined for the presence of haemadsorption. The method described in the EP would be acceptable.

2.3.4. Examination for specified viruses.

For viruses which are unlikely to be detected by the general tests described above, specific test methods must be applied. These are indicated under the heading “Specific tests” in the relevant tables.

Sufficient cells ***( to be defined)*** on suitable supports ***( to be defined)*** are prepared to carry out tests for the agents specified. The material shall be obtained after at least 2 passages and a surface of at least 6 sqcm shall be examined. Suitable positive controls and negative controls are included in each test. The cells are subjected to suitable tests, for example using immunochemical methods as e.g. fluorescein-conjugated antibodies or other techniques, especially those suitable for testing large number of samples. .***( to be discussed)***

**2.2.Test for the presence of extraneous viruses in cell stocks**

2.2.1. General.

**Note:**

**The present text of the guideline applies to veterinary viral vaccines prepared in established cell-lines only.**

Normally, wherever possible, the cell stocks are prepared and maintained in accordance with the seed-lot principle.

It is normally required that the cell stock is tested to the extent possible for the presence of extraneous agents. Tests should be carried out for possible contaminating viruses that may originate from:

- the source species of the material.
- from those animal species to which the cells material may have been exposed
-

and also

- for the presence of possible contaminating viruses that may occur in the animal species for which the finished product is intended.

[*The test is done on the Master Cell Stock and on the maximum passage level of the Master Cell Stock used in production , normally not exceeding the 20th passage. Higher levels may be acceptable, provided adequate data are provided to support the suitability of this higher passage level.*]

2.2.2. Samples

The samples shall originate from well-defined seedlots only.

The size of the sample shall depend on the number of cells present in the cell seed material but shall contain at least $1 \times 10^5$ cells per ml.
Alternatively monolayers with a total surface of at least 75 cm2 are required.
The size of the sample taken in the case of suspension cultures shall be at least $1x10^5$ cells per ml. or at least 1 ml. of the suspension culture, whichever sample contains the largest number of cells.

2.2.3. Substrates

The substrates used for testing the absence of extraneous agents shall consist of at least three different types of sensitive cells and include at least:

- primary cells of the source species;
- cells sensitive to viruses pathogenic for the species for which the vaccine is intended;
- cells sensitive to pestiviruses. The sensitivity of these cells shall be demonstrated by the use of positive controls e.g. Bovine Viral Diarrhoea virus.

2.2.4. Test method.

The test to determine the absence of extraneous agents in cells consists of two different stages:

- the test on the initial cell-cultures
- the test on the passaged cell-cultures

2.2.4.1. The test on the initial cell-cultures.

2.2.4.1.1. Preparation of the cell cultures.

The Master Cell Stock is used to establish monolayers in suitable culture vessels.

The monolayers to be used in the test shall cover an area of at least 75 $cm^2$ and shall be prepared and maintained using medium and additives, and grown under similar conditions – as far as is feasible - to those used for the preparation of the vaccine. At least the culture-media, the cell-system and incubation temperature shall be mimicked.

2.2.4.1.2. Procedure.

At least 2 subcultures are made of the monolayers with an interval of 7 days. The cell-cultures are maintained for at least 21 (28) days during which period the cultures are examined regularly for cytopathogenic effect.

At least 7 days after the initiation of the last subculture they are examined :

- for the presence of haemadsorbtion.
- microscopically for cytopathogenic effect, inclusion bodies etc. by staining of 2 monolayers with May Grunwald Giemsa, HE or other suitable staining methods.
- for the presence of specific extraneous agents by means of immunofluorescent antibodies.

For details see 2.1.4.3.

2.2.4.2.The test on the passaged cell-cultures.

2.2.4.2.1. Preparation of the cell cultures.

The Master Cell Stock is used to establish monolayers with a surface of at least 140 $cm^2$.

2.2.4.2.2. Procedure.

The cells of these monolayers are subjected to freezing-thawing ( 3 times). The cell material is centrifuged at >2000 g for > 15 minutes and the sediment is collected.

The sediment (1 ml) is inoculated on each of two monolayers of each of the cell cultures, with a surface of > 75 $cm^{2.}$,

One subculture is made with an interval of 7 days. The cell-cultures are maintained for at least 14 days during which period the cultures are examined regularly for cytopathogenic effect.

At least 7 days after the initiation of the subculture the cells are examined:

- for the presence of haemadsorbtion.
- microscopically for cytopathogenic effect, inclusion bodies etc. by staining 2 monolayers with May Grunwald Giemsa, HE or other suitable staining methods.
- for the presence of specific extraneous agents by means of immunofluorescent antibodies.

For details see 2.1.4.3.

## 2.3. Test for the presence of extraneous viruses in materials of animal origin.

2.3.1. General .

Normally the material of animal origin shall be tested before it is subjected to a sterilsation procedure. The material is tested by applying tests of a general nature that may be expected to detect a broad selection of agents ,complemented by specific tests to detect individual agents for which there is a particular risk of occurrence. These agents - only of viral nature - are listed in Annex 2.

2.3.2. Samples.

The sample size c.q. the volume of material used for testing shall normally be related to the quantity of the material used in the production process and to the size of the total quantity of the batch of starting material to be tested.

The material, when prepared for testing, shall be dissolved or suspended in a defined medium, that is identical or at least related to the medium in which the substance will be used in the production process, provided this medium is compatible with the test system.

Any solids are dissolved or suspended in a suitable medium ***( to be defined)*** in such a way as to create a solution or suspension containing at least 30 per cent w/v of the substance to be examined.

If the substance is not soluble or where cytotoxic reactions occur, a lower concentration may be used. ***( to be defined)***

2.3.3. Substrates.

2.3.4. Test method.

2.3.4.1. Preparation of substrate cells.

2.3.4.2. Inoculum.

2.3.4.3. Procedure.

## 3. GLOSSARY

**BATCH ( STARTING MATERIAL OF ANIMAL ORIGIN)**

The total quantity of the material mixed in a single container and identified by a serial number.

**BATCH (FINAL LOT) VACCINE**

A collection of closed, final containers or other final dosage units that are expected to be homogeneous and equivalent with respect to risk of contamination during filling or preparation of the final product. The dosage units are filled, or otherwise prepared, from the same final bulk vaccine, freeze-dried together (if applicable) and closed in one continuous working session. They bear a distinctive number or code identifying the final lot (batch). Where a final bulk vaccine is filled and/or freeze-dried on several separate sessions, there results a related set of final lots (batches) that are usually identified by the use of a common part in the distinctive number or code; these related final lots (batches) are sometimes referred to as sub-batches, sub-lots or filling lots.

**CELL-SEED SYSTEM**

A system whereby successive final lots (batches) of a product are manufactured by culture in cells derived from the same master cell seed. A number of containers from the master cell seed are used to prepare a working cell seed.

**CELL LINES**

Cultures of cells that have a high capacity for multiplication *in-vitro.*

**CELL PASSAGE**

The result of an *in-vitro* process whereby cells are replicated, either by being transferred from one container to another or provided with additional growth surface or volume intended to result in renewed or expanded cell growth.

**CONTROL CELLS**

A quantity of cells set aside, at the time of inoculation, as uninfected cell cultures and which are the same as used in the test. The uninfected cells are incubated under similar conditions to those used for the test

**MASTER CELL SEED**

A collection of aliquots of cells for use in the preparation of the product, distributed into containers in a single operation, processed together and stored in such a manner as to ensure uniformity and stability and to prevent contamination. Master cell seed is usually stored at temperatures of - 70 °C or lower.

**MASTER SEED LOT**

A culture of a micro-organisms for use in the preparation of the product, distributed from a single bulk into containers and processed together in a single operation in such a manner as to ensure uniformity and stability and to prevent contamination.

**PRIMARY CELL CULTURES**

Primary cell cultures are cultures of cells essentially unchanged from those in the animal tissues from which they have been prepared and being no more than 10 *in-vitro* passages to the test level from the initial preparation from the animal tissue. The first *in-vitro* cultivation is regarded as the first passage of the cells.

**SEED-LOT SYSTEM:**

A seed-lot system is a system according to which successive batches of a product are derived from the same master seed virus. For routine production, a working seed virus may be prepared from the master seed virus.

**VIRUS PASSAGE**

The result of an *in-vitro* or *in-vivo* process whereby a virus is replicated, either by inoculation of non-infected cells, embryos or animals or performing a cell passage in the case of persistently infected cells.

**WORKING CELL SEED**

A collection of aliquots of cells derived from the master cell seed and intended for use in the preparation of production cell cultures. The working cell seed is distributed into containers, processed and stored as described for master cell seed.

**WORKING SEED VIRUS**

A collection of aliquots of a micro-organism derived from the master seed virus and intended for use in production. Working seed virus is distributed into containers and stored as described for master seed virus.

## 4. ANNEXES

### 4.1. LIST OF EXTRANEOUS AGENTS

The extraneous agents which must be taken into account when considering which extraneous agents have to be tested for are listed in the tables, according to animal species.

The presence of an agent shall be determined as matter of principle. The presence of an agent in the table does not necessarily mean that a specific test for that agent must be carried out, but a justification for not carrying out a specific test for an agent will be required.

The types of justification that can be given include:

a) Disease does not occur in country of origin (and material could not have been contaminated by this agent, subsequently). Supporting documentary evidence must be provided.
b) Substance In question cannot be contaminated with this agent, e.g. agent does not cross placenta.
c)The applicant has shown that the general test can be shown to detect the specified agent, with equivalent sensitivity.

For points (a) and (b), these justifications may be given by referring to published literature.

In addition to consideration of relevance of the agents listed In the table,changes in the disease situation in the country of origin of materials and, particularly, new emerging disease shall be taken into account.

It has been remarked that TSE agents are not included in the list and that it might be necessary to apply such lengthy tests retroactively when the need for testing is justified.

able 1 Extraneous agents relevant for bovines and for material of bovine origin.

| AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|
| | | GENERAL TESTS | SPECIFIC TESTS |
| | | | |
| Adenovirus , subgroups 1 and 2 * | + | + | |
| Akabane virus | + | | + |
| Aujeszky's Disease virus | + | + | |
| Bluetongue virus | + | | + |
| Epizootic Haemorrhagic Disease virus | + | | + |
| Bovine Corona virus | + | + | |
| Bovine Ephemeral Fever virus | + | | |
| Bovine Herpes virus type 1, 2 and 4 | + | + | |
| Bovine Leukaemia virus | + | | + |
| Bovine Papilloma virus | + | | |
| Bovine Parvo virus | + | + | |
| Bovine Papular Stomatitis virus - pseudocowpox virus | + | | |
| Bovine Respiratory Syncytial virus | + | | |
| Bovine Rota virus | + | | |
| Bovine Viral Diarrhoea virus | + | | + |
| Cowpox virus , vaccinia virus | + | + | + |
| Foot and Mouth Disease virus types A,O,C, Asia 1, SAT 1, SAT 2, SAT 3 | + | | + |

| AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|
| | | GENERAL TESTS | SPECIFIC TESTS |
| Lumpy Skin Disease virus | + | + | |
| Malignant Catarrhal Fever ( African form) virus | + | | |
| Malignant Catarrhal Fever ( European form) virus | + | | |
| Parainfluenza 3 virus | + | + | |
| Coxiella burnetti ( Q-fever) | + | + | |
| Rabies virus | + | | + |
| Rift Valley Fever virus | + | | + |
| Rinderpest virus | + | + | |
| Vesicular Stomatitis Virus ( Indiana and New Yersey) | + | + | |
| ***Bovine polyoma virus*** | | | |

* *Sensitivity of tests on Bovine Adenovirus subtype 2.: only grows on primary calf testes cells.*

Table 2 Extraneous agents relevant for ovines and caprines and of material of ovine or caprine origin.

| SPECIES | AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|---|
| | | | GENERAL TESTS | SPECIFIC TESTS |
| | | | | |
| O + C | Adenovirus , subgroups 1 and 2 | + | + | |
| O + C | Akabane virus | + | | + |
| O + C | Aujeszky's Disease virus | + | + | |
| O + C | Bluetongue virus | + | | + |
| | Epizootic haemorrhagic Disease virus | + | | + |
| O + C | Bovine Herpes virus type 1, 2 and 4 | + | + | |
| O | Bovine Leukaemia virus | + | | + |
| O | Bovine Papilloma virus | + | | |
| O | Bovine Viral Diarrhoea virus | + | | + |
| O + C | Cowpox virus , vaccinia virus | + | + | + |
| O + C | Foot and Mouth Disease virus types A,O,C, Asia 1, SAT 1, SAT 2, SAT 3 | + | | + |
| O + C | Parainfluenza 3 virus | + | + | |
| O + C | Rift Valley Fever virus | + | | + |
| C | Caprine Arthritis Encephalitis virus | + | | + |
| C | Caprine Herpes virus | + | + | |
| O | Border Disease virus | + | | + |
| O | Borna Disease virus | + | | + |

| SPECIES | AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|---|
| | | | GENERAL TESTS | SPECIFIC TESTS |
| O | Ovine Pulmonary Adenomatosis virus | + | | |
| O | Louping Ill virus | + | | + |
| O | Nairobi Sheep Disease virus | + | | + |
| O | Ross River virus | + | + | |
| O | Scrapie * | + | | |
| O + C | Ecthyma contagiosum virus ( ORF virus) | + | + | |
| O + C | Maedi Visna virus | + | | |
| O + C | Peste des petits ruminants virus | + | + | |
| | | | | |

O : Ovine
C : Caprine

* : Demonstration of freedom of scrapie requires monitoring for absence of disease for at least two years and examination of brain material from culled animals by histology and/or examination for scrapie associated fibrils.

Table 3 Extraneous agents relevant for porcines and of material of porcine origin.

| AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|
| | | GENERAL TESTS | SPECIFIC TESTS |
| | | | |
| African Swine Fever virus | + | | + |
| Aujeszky's Disease virus | + | + | |
| Bovine Viral Diarrhoea virus | + | | + |
| Classical Swine Fever virus | + | | + |
| Encephalomyocarditis virus | + | + | |
| Foot and Mouth Disease virus types A,O,C, Asia 1, SAT 1, SAT 2, SAT 3 | + | | + |
| Haemagglutinating encephalomyelitis virus | + | + | |
| Transmissible Gastro Enteritis virus and Porcine Respiratory Corona virus | + | + | |
| Porcine Adeno viruses | + | + | |
| Porcine Cytomegalo virus | + | | + |
| Porcine Epidemic Diarrhoea virus | + | | |
| Porcine Entero viruses ( Incl. Teschen-Talfan virus) | + | + | |
| Porcine Influenza virus | + | | + |
| Porcine Parvo virus | + | | + |
| Porcine Respiratory and Reproductive Syndrome virus | + | | + |
| Porcine Rota virus | + | | |
| Porcine Vesicular Exanthema virus | + | + | |

| AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|
| | | GENERAL TESTS | SPECIFIC TESTS |
| Rabies virus | + | | + |
| Swine Pox virus | + | + | |
| Swine Vesicular Disease virus | + | + | |
| Vesicular Stomatitis virus | + | | + |
| ***RETROVIRUSES (??) more information required*** | | | |

Table 4 Extraneous agents relevant for equines and of material of equine origin.

| AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|
| | | GENERAL TESTS | SPECIFIC TESTS |
| | | | |
| African Horse Sickness virus | + | | + |
| Borna Disease virus * | + | | |
| Equine Arteritis virus | + | + | |
| Equine encephalomyelitis virus ( Eastern, Western, Venezuelian) | + | | + |
| Equine Herpes virus types 1, 2 , 3 and 4 | + | + | |
| Equine Infectious Anaemia virus | + | | + |
| Equine Influenza virus | + | | + |
| Japanese B. Encephalitis virus | + | | + |
| Rabies virus | + | | + |
| Vesicular Stomatitis virus | + | | + |

** Borna disease virus can only be detected by intracerebral inoculation of rabbits or new-born rats*

Table 5 Extraneous agents relevant for felines and of material of feline origin.

| AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|
| | | GENERAL TESTS | SPECIFIC TESTS |
| | | | |
| Aujeszky's Disease virus | + | + | |
| Cowpox virus | + | + | |
| Feline Calici virus | + | + | |
| Feline Herpes virus type 1 | + | + | |
| Feline Immunodeficiency virus | + | | + |
| Feline Leukaemia virus / Feline Sarcoma virus | + | | + |
| Feline Infectious Peritonitis virus / Feline Enteric Corona virus | + | | |
| Feline Panleucopenia virus | + | + | |
| Feline Syncytia Forming virus | + | | + |
| Rabies virus | + | | + |

Table 6 Extraneous agents relevant for canines and of material of canine origin.

| AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|
| | | GENERAL TESTS | SPECIFIC TESTS |
| | | | |
| Aujeszky's Disease virus | + | + | |
| Canine Adeno virus type 1 and 2 | + | + | |
| Canine Corona virus | + | + | |
| Canine Distemper virus | + | + | |
| Canine Herpes virus | + | + | + |
| Canine Parvo virus | + | + | + |
| Parainfluenza 2 virus ( SV-5) | + | + | |
| Rabies virus | + | | + |

Table 7 Extraneous agents relevant for rabbits and of material of rabbit origin.

| AGENT | *AGENTS THAT HAVE TO BE ABSENT IN SPF HERDS* | *TESTS TO BE USED TO DEMONSTRATE ABSENCE OF THE AGENTS IN CELLS AND SEEDS* | |
|---|---|---|---|
| | | GENERAL TESTS | SPECIFIC TESTS |
| | | | |
| Arena virus ( Lymphocytic Choriomeningitis virus) | + | | + |
| Aujeszky's Disease virus | + | + | |
| Encephalomyocarditis virus | + | + | |
| Myxoma virus ( Shope Fibroma virus) | + | | + |
| Rabbit Haemorrhagic Disease virus | + | - | + |
| Rabies virus | + | | + |

Table 8. Extraneous agents to be absent in VERO cells.

**QUESTION:**

**It was considered not to be necessary to test well-known cell lines with a very long history for absence of viruses in primary cells of the species of origin.**

**DECISION:**

NOTE:

During the meeting of the Working Group in Brussels in November 1999 the necessity to include Bovine Polyma Virus in the list of viruses for which substances of bovine origin shall be tested was discussed. It was decided to collect some more information on this virus in order to make it possible to come to a definite opinion.

Dr. Peter Castle, of the European Pharmacopoeia Commission made available the following 3 scientific papers on BPyV:


1. Noorda, J.van der et al.
   Bovine Polyomavirus, a frequent contaminant of calf sera.
   Animal Sera Derivatives and Substitutes used in the manufacturing of Pharmaceuticals. (Ed. Brown F et al)
   Dev.Biol.Stand. Basel Karger 1999, 99, 45-47

2. Kappeler,A et al.
   Detection of Bovine Polyomavirus contamination in fetal bovine serra and modified live viral vaccines using polymerase chain reaction
   Biologicals 1996, 24, 131-135

3. Schuurman R, et al.
   Bovine Polyomavirus, a frequent contaminant of calf serum
   Biologicals 1991, 19, 265-270

The information in these publications,as far as relevant to the discussion, can be summarized as follows:

- BPyV, which belongs to the genus of the Papovaviruses, does occur in a large part (40-60%) of the bovine populaton, but does not appear to be associated with any clinical signs.
- The infection rate of cattle in the USA and Europe appears to be similar. Commercial batches of bovine serum from these two regions had an contamination rate of approximately 60-70%.
- BPyV causes a persistent infection in bovines and the virus may pass the pacental barrier and infect the fetus.
- Bovine serum, either of fetal origin or obtained from new born or adult cattle is frequently contaminated with BpyV.
- BPyV cannot be removed from the serum by filtration because of its small size. Using a filter with a smaller pore-size is not a realistic option because such filter will be clogged by the serum proteins
- BPyV is difficult to inactivate by physico-chemical methods inactivation methods e.g. irradiation, heat and solvents as ether and chloroform.
- BPyV is able to infect cell cultures obtained fron non-bovine sources e.g. monkeys(including VERO cells), humans, dogs
- BPyV antibodies do occur frequently in humans closely associated with animals as e.g veterinarians, farmers, slaughterhouse personell.
- The presence of BPyV in bovine serum cannot be excluded on the basis of testing for antibodies alone, because such a test will not detect the presence of free circulating virions.

The PCR test seems to be the method of choice to detect the presence of BPyV.

Note:

*In the case it is decided to include bovine polyoma virus in the list of agents for which substances of bovine origin shall be tested, it would be logical to stipulate that all substance of animal origin, prepared by a method involving the use of bovine serum should be tested for the presence of the virus as well.*

## 4.2. Present national requirements and proposal for harmonisation.

European Pharmacopoeia - Monograph 62 - Vaccines for Veterinary Use (1995)
CVMP - General requirements for the production and control of live mammalian bacterial and viral vaccines for veterinary use (1993)
CVMP - General requirements for the production and control of inactivated mammalian bacterial and viral vaccines for veterinary use (1993)
CVMP - Note for Guidance - Table of extraneous agents to be tested for in relation to the general and species specific guidelines on the Production and control of mammalian veterinary vaccines (1993)
Code of Federal Regulations (1996)
9CFR 113.55 Detection of extraneous agents in Master Seed Virus
9CFR 113.46 Detection of cytopathogenic and/or haemadsorbing agents
9CFR 113.47 Detection of extraneous viruses by the fluorescent antibody technique
9CFR 113.51 Requirements for primary cells used for production of biologics
9CFR 113.52 Requirements for cell lines used for production of biologics
Japanese requirements for testing of vaccines for extraneous agents - Intervet K.K. (personal communication)